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Title of the Invention

CIRCOVIRUS SEQUENCES ASSOCIATED WITH PIGLET WEIGHT LOSS DISEASE (PWD)

Information on Related Applications

The present application claims the priority benefit, under 35 U.S.C. § 119, of International Application No. PCT/FR98/02634, filed ~~{January 23}~~ **[December 4]**, 1998.

Background of the Invention

The invention relates to the genomic sequence and nucleotide sequences coding for polypeptides of PWD circovirus, such as the structural and nonstructural polypeptides of said circovirus, as well as vectors including said sequences and cells or animals transformed by these vectors. The invention likewise relates to methods for detecting these nucleic acids or polypeptides and kits for diagnosing infection by the PWD circovirus. The invention is also directed to a method for selecting compounds capable of modulating the viral infection. The invention further comprises pharmaceutical compositions, including vaccines, for the prevention and/or the treatment of viral infections by PWD circovirus as well as the use of a vector according to the invention for the prevention and/or the treatment of diseases by gene therapy.

Piglet weight loss disease (PWD), alternatively called fatal piglet wasting (FPW) has been widely described in North America (Harding, J.C., 1997), and authors have reported the existence of a relationship between this pathology and the presence of porcine circovirus (Daft, B. et al., 1996; Clark, E.G., 1997; Harding, J.C., 1997; Harding, J.C. and Clark, E.G., 1997; Nayar, G.P. et al., 1997). A porcine circovirus has already been demonstrated in established lines of cell cultures derived from pigs and chronically infected (Tischer, I., 1986, 1988, 1995; Dulac, G.C., 1989; Edwards, S., 1994; Allan, G.M., 1995 and McNeilly, F., 1996). This

virus, during experimental infection of piglets, does not prove pathogenic for pigs (Tischer, I., 1986, Horner, G.W., 1991) and its nucleotide sequence has been determined and characterized (Tischer, I., 1982; Meehan, B.M. et al., 1997; Mankertz., A., 1997). The porcine circovirus, called PCV virus, is part of the circovirus genus of the circoviridae family (Murphy, F.A. et al., 1995) whose virion has a circular DNA of size between 1.7 and 2.3 kb, which DNA comprises three open reading frames (ORF1 to ORF3), coding for a replication protein REP involved in the initiation and termination phase of rolling circular replication (RCR) (Heyraud-Nitschke, F., et al., 1995; Harding, M.R. et al., 1993; Hanson, S.F. et al., 1995; Fontes, E.P.B. et al., 1994), coding for a capsid protein (Boulton, L.H. et al., 1997; Hackland, A.F. et al., 1994; Chu, P.W.G. et al., 1993) and coding for a nonstructural protein called a dissemination protein (Lazarowitz., S.G. et al., 1989).

The authors of the present invention have noticed that the clinical signs perceptible in pigs and linked to infection by the PWD circovirus are very distinctive. These manifestations in general appear in pigs of 8 to 12 weeks of age, weaned for 4 to 8 weeks. The first signs are hypotonia without it being possible to speak of prostration. Rapidly (48 hours), the flanks hollow, the line of the spine becomes apparent, and the pigs "blanch." These signs are in general accompanied by hyperthermia, anorexia and most often by respiratory signs (coughing, dyspnea, polypnea). Transitory diarrhea can likewise appear. The disease state phase lasts approximately one month at the end of which the rate of mortality varies from 5 to 20%. To these mortalities, it is expedient to add a variable proportion (5-10%) of cadaveric animals which are no longer able to present an economic future. It is to be noted that outside of this critical stage of the end of post-weaning, no anomaly appears on the farms. In particular, the reproductive function is totally maintained.

On the epidemiological level, the first signs of this pathology appeared at the start of 1995 in the east of the Côtes d'Armor region in France, and the farms affected are especially confined to this area of the region. In December 1996, the

number of farms concerned could not be evaluated with precision because of the absence of a specific laboratory diagnostic method or of an epidemioligical surveillance system of the livestock. Based on the clinical facts as well as on results of postmortem examinations supplied by veterinarians, it is possible to estimate this number as several dozen (80-100). The contagiousness of the disease is weak to moderate. Cases are being reported outside the initial area and for the majority are following the transfer of animals coming from farms familiar with the problem. On the other hand, a characteristic of the condition is its strong remanence. Thus, farms which have been affected for a year are still affected in spite of the massive administration of therapeutics. Farms with clinical expression are drawn from various categories of specialization (breeders/fatteners, post-weaners/ fatteners) and different economic structures are concerned. In addition, the disorders appear even in farms where the rules of animal husbandry are respected.

Numerous postmortem examinations have been carried out either on farms or in the laboratory. The elements of the lesional table are disparate. The most constant macroscopic lesions are pneumonia which sometimes appears in patchy form as well as hypertrophy of the lymphatic ganglia. The other lesions above all affect the thoracic viscera including, especially, pericarditis and pleurisy. However, arthritis and gastric ulcers are also observed. The lesions revealed in the histological examination are essentially situated at the pulmonary level (interstitial pneumonia), ganglionic level (lymphoid depletion of the lymph nodes, giant cells) and renal level (glomerulonephritis, vasculitis). The infectious agents have been the subject of wide research. It has been possible to exclude the intervention of pestiviruses and Aujeszky's disease. The disorders appear in the seropositive PDRS (Porcine Dysgenic and Respiratory Syndrome, an infection linked to an arteriovirus) herds, but it has not been possible to establish the role of the latter in the genesis of the disorders (the majority of the farms in Brittany are PDRS seropositive).

The authors of the present invention, with the aim of identifying the etiological agent responsible for PWD, have carried out "contact" tests between

piglets which are obviously "ill" and SPF pigs (specific pathogen-free) from CNEVA (Centre National d'Etudes Vétérinaires et Alimentaires, France). These tests allow the development of signs comparable to those observed on the farm to be observed in protected animal houses. The discrete signs such as moderate hyperthermia, anorexia and intermittent diarrhea appeared after one week of contact. It must be noted that the PDRS virus only diffused subsequent to the clinical signs. In addition, inocculations of organ homogenates of sick animals to healthy pigs allowed signs related to those observed on the farms to be reproduced, although with a lower incidence, linked to the favorable conditions of upkeep of the animals in the experimental installations.

Thus, the authors of the present invention have been able to demonstrate that the pathological signs appear as a well-defined entity affecting the pig at a particular stage of its growth.

This pathology has never been described in France. However, sparse information, especially Canadian, relates to similar facts.

The disorders cannot be mastered with the existing therapeutics.

The data collected both on the farm and by experimentation have allowed the following points to be higlighted:

- PWD is transmissible but its contagiousness is not very high,
- its etiological origin is of infectious and probably viral nature,
- PWD has a persistent character in the affected farms.

Considerable economic consequences ensue for the farms.

Thus, there is currently a significant need for a specific and sensitive diagnostic, whose production is practical and rapid, allowing the early detection of the infection.

A reliable, sensitive and practical test which allows the distinction between strains of porcine circovirus (PCV) is thus strongly desirable.

On the other hand, a need for efficient and well-tolerated treatment of infections with PWD circovirus likewise remains desirable, no vaccine currently being available against PWD circovirus.

Concerning PWD circovirus, it will probably be necessary to understand the role of the immune defense in the physiology and the pathology of the disease to develop satisfactory vaccines.

Fuller information concerning the biology of these strains, their interactions with their hosts, the associated infectivity phenomena and those of escape from the immune defenses of the host especially, and finally their implication in the development of associated pathologies, will allow a better understanding of these mechanisms. Taking into account the facts which have been mentioned above and which show in particular the limitations of combatting infection by the PWD circovirus, it is thus essential today on the one hand to develop molecular tools, especially starting from a better genetic knowledge of the PWD circovirus, and likewise to perfect novel preventive and therapeutic treatments, novel methods of diagnosis and specific, efficacious and tolerated novel vaccine strategies. This is precisely the subject of the present invention.

Summary of the Invention

[The present invention relates to vaccines comprising a nucleotide sequence of the genome of Porcine circovirus type B, or a homologue or fragment thereof, and an acceptable pharmaceutical or veterinary vehicle. In one embodiment of the invention, the nucleotide sequence is selected from SEQ ID No. 15, SEQ ID No. 19 SEQ ID No. 23, or SEQ ID No. 25, or a homologue or fragment thereof. In another embodiment of the invention, the homologue has at least 80% sequence identity to SEQ ID No. 15, SEQ ID No. 19, SEQ ID No. 23 or SEQ ID No. 25. In yet another embodiment, the vaccines further comprising an adjuvant

The present invention also relates to vaccines comprising a polypeptide encoded by a nucleotide sequence of the genome of PCVB, or a homologue or fragment thereof, and an acceptable pharmaceutical or veterinary vehicle. In one embodiment, the homologue has at least 80% sequence identity to SEQ ID No. 15, SEQ ID No. 19, SEQ ID No. 23 or SEQ ID No. 25. In another embodiment of the invention, the nucleotide sequence is selected from SEQ ID No. 23 or SEQ ID No. 25, or a homologue or fragment thereof. In still another embodiment, the polypeptide has the amino acid sequence of SEQ ID No. 24 or SEQ ID No. 26. In yet another embodiment, the homologue has at least 80% sequence identity to SEQ ID No. 24 or SEQ ID No. 26. In another embodiment, the polypeptide has the amino acid sequence of SEQ ID No. 29, SEQ ID No. 30, SEQ ID No. 31, or SEQ ID No. 32.

A further aspect of the invention relates to vaccines comprising a vector and an acceptable pharmaceutical or veterinary vehicle, the vector comprising a nucleotide sequence of the genome of Porcine circovirus type B, or a homologue or fragment thereof. In one embodiment, the vaccine further comprises a gene coding for an expression product capable of inhibiting or retarding the establishment or development of a genetic or acquired disease.

The present invention also relates to vaccines comprising a cell and an acceptable pharmaceutical or veterinary vehicle, wherein the cell is transformed with a nucleotide sequence of the genome of Porcine circovirus type B, or a homologue or fragment thereof.

Still further, the present invention relates to vaccines comprising a pharmaceutically acceptable vehicle and a single polypetide, wherein the single polypeptide consists of SEQ ID No. 26.

Additionally, the present invention relates to methods of immunizing a mammal against piglet weight loss disease comprising administering to a mammal an effective amount of the vaccines desribed above.

These and other aspects of the invention will become apparent to the skilled artisan in view of the teachings contained herein.]

Brief Description of the Drawings

Figure 1: Experimental scheme which has made it possible to bring about the isolation and the identification of the circovirus associated with PWD of type A and B.

Test 1: experimental reproduction of the PWD by inoculation of pig organ homogenates from farms affected by PWD.

Test 2: experimental reproduction of PWD.

Test 3: experimental reproduction of PWD.

Test 4: no experimental reproduction of PWD.

Figure 2: Organization of the genome of the circovirus associated with PWD of type A (PCVA)

- strand of (+) polarity (SEQ ID No. 1);
- strand of (-) polarity (SEQ ID No. ~~{2}~~ **[5]**, represented according to the orientation 3' → 5');
- sequences of amino acids of proteins encoded by the two DNA strands in the three possible reading frames **[SEQ ID NOS: 2-4 and 6-8 respectively]**.

Figure 3: Alignment of the nucleotide sequence SEQ ID No. 1 of the PWD circovirus of type A (PCVA) and of the MEEHAN **[SEQ ID No. 163]** strain and MANKERTZ **[SEQ ID No. 164]** strain circoviruses of the porcine cell lines.

Figure 4: Alignment of the sequence of amino acids SEQ ID No. ~~{6}~~ **[10]** of a polypeptide encoded by the nucleotide sequence SEQ ID No. ~~{3}~~ **[9]** (ORF1) of the PWD circovirus of type A (PCVA) and of corresponding nucleotide sequences

of the MEEHAN **[SEQ ID No. 165]** strain and MANKERTZ **[SEQ ID No. 166]** strain circoviruses of the porcine cell lines.

Figure 5: Alignment of the sequence of amino acids SEQ ID No. ~~{7}~~ **[12]** of a polypeptide encoded by the nucleotide sequence SEQ ID No. ~~{4}~~ **[11]** (ORF2) of the PWD circovirus of type A (PCVA) and of corresponding nucleotide sequences of the MEEHAN **[SEQ ID No. 167]** strain and MANKERTZ **[SEQ ID No. 168]** strain circoviruses of the porcine cell lines.

Figure 6: Alignment of the sequence of amino acids SEQ ID No. ~~{8}~~ **[14]** of a polypeptide encoded by the nucleotide sequence SEQ ID No. ~~{5}~~ **[13]** (ORF3) of the PWD circovirus of type A (PCVA) and of corresponding nucleotide sequences of the MEEHAN **[SEQ ID No. 169]** strain and MANKERTZ **[SEQ ID No. 170]** strain circoviruses of the porcine cell lines.

Figure 7: Western blot analysis of recombinant proteins of the PWD circovirus of type A (PCVA).

The analyses were carried out on cell extracts of Sf9 cells obtained after infection with recombinant baculovirus PCF ORF 1.

Figure 8: Organization of the genome of the circovirus associated with the PWD of type B (PCVB)

- strand of (+) polarity (SEQ ID No. ~~{9}~~) **[15]**;
- strand of (-) polarity (SEQ ID No. ~~{10}~~ **[19]**, represented according to the orientation 3' → 5');
- sequence of amino acids of proteins encoded by the two DNA strands in the three possible reading frames **[SEQ ID NOS: 16-18 and 20-22 respectively]**.

Figure 9: Evolution of the daily mean gain (DMG) of pig farms affected by piglet weight loss disease (PWD), placed under experimental conditions.

Figure 10: DMG compared for the 3 batches of pigs (F1, F3 and F4) calculated over a period of 28 days, after vaccination test.

Figure 11: Hyperthermia greater than 41°C, expressed as a percentage compared for the 3 batches of pigs (F1, F3 and F4) calculated per week over a period of 28 days, after vaccination test.

Figure 12: Membranes of peptide spots corresponding to the ORF2s revealed with the aid of an infected pig serum, originating from a conventional farm.

The numbers of specific peptides of the circovirus of type B as well as their nonreactive homologs (type A) are indicated in bold.

The nonspecific immunogenic peptides are indicated in italics.

Figure 13: Alignment of amino acid sequences of proteins encoded by the ORF2 of the PWD circovirus of type A **[SEQ ID No. 12]** and by the ORF'2 of the PWD circovirus of type B **[SEQ ID No. 26]**. The position of 4 peptides corresponding to specific epitopes of the PWD circovirus of type B is indicated on the corresponding sequence by a bold line, their homolog on the sequence of the PWD circovirus of type A is likewise indicated by an ordinary line.

Figure 14: Charts the results of experiments that demonstrate, in terms of percent hyperthermia, that vaccination with ORF'1 and ORF'2 of PCV-B enhances the level of protection in swine challeneged with PCV-B.

Figure 15: Charts the results of experiments that demonstrate, in terms of animal growth, that vaccination with ORF'1 and ORF'2 of PCV-B enhances the level of protection in swine challeneged with PCV-B.

Figure 16: Immunoperoxidase staining of PK15 cells at 24 h post-transfection with the pcDNA3/ORF'2 plasmid. Expression of PCVB ORF'2 was confirmed by IPMA following incubation in the presence of the swine anti-PCVB monospecific serum

Detailed Description of the Invention

The present invention relates to nucleotide sequences of the genome of PWD circovirus selected from the sequences SEQ ID No. 1, SEQ ID No. ~~{2}~~ **[5]**, SEQ ID No. ~~{9}~~ **[15]**, SEQ ID No. ~~{10}~~ **[19]** or one of their fragments.

The nucleotide sequences of sequences SEQ ID No. 1 and SEQ ID No. ~~{2}~~ **[5]** correspond respectively to the genome sequence of the strand of (+) polarity and of the strand of (-) polarity of the PWD circovirus of type A (or PCVA), the sequence SEQ ID No. ~~{2}~~ **[5]** being represented according to the orientation 5'→3'.

The nucleotide sequences of sequences SEQ ID No. ~~{9}~~ **[15]** and SEQ ID No. ~~{10}~~ **[19]** correspond respectively to the genome sequence of the strand of (+) polarity and of the strand of (-) polarity of the PWD circovirus of type B (or PCVB), the sequence SEQ ID No. ~~{10}~~ **[19]** being represented according to the orientation 5'→3'.

The present invention likewise relates to nucleotide sequences, characterized in that they are selected from:

a) a nucleotide sequence of a specific fragment of the sequence SEQ ID No. 1, SEQ ID No. ~~{2}~~ **[5]**, SEQ ID No. ~~{9}~~ **[15]**, SEQ ID No. ~~{10}~~ **[19]** or one of their fragments;

b) a nucleotide sequence homologous to a nucleotide sequence such as defined in a);

c) a nucleotide sequence complementary to a nucleotide sequence such as defined in a) or b), and a nucleotide sequence of their corresponding RNA;

d) a nucleotide sequence capable of hybridizing under stringent conditions with a sequence such as defined in a), b) or c);

e) a nucleotide sequence comprising a sequence such as defined in a), b), c) or d); and

f) a nucleotide sequence modified by a nucleotide sequence such as defined in a), b), c), d) or e).

Nucleotide, polynucleotide or nucleic acid sequence will be understood according to the present invention as meaning both a double-stranded or single-stranded DNA in the monomeric and dimeric (so-called in tandem) forms and the transcription products of said DNAs.

It must be understood that the present invention does not relate to the genomic nucleotide sequences taken in their natural environment, that is to say in the natural state. It concerns sequences which it has been possible to isolate, purify or partially purify, starting from separation methods such as, for example, ion-exchange chromatography, by exclusion based on molecular size, or by affinity, or alternatively fractionation techniques based on solubility in different solvents, or starting from methods of genetic engineering such as amplification, cloning and subcloning, it being possible for the sequences of the invention to be carried by vectors.

The nucleotide sequences SEQ ID No. 1 and SEQ ID No. ~~{9}~~ **[15]** were obtained by sequencing of the genome by the Sanger method.

Nucleotide sequence fragment according to the invention will be understood as designating any nucleotide fragment of the PWD circovirus, type A or B, of length of at least 8 nucleotides, preferably at least 12 nucleotides, and even more preferentially at least 20 consecutive nucleotides of the sequence from which it originates.

Specific fragment of a nucleotide sequence according to the invention will be understood as designating any nucleotide fragment of the PWD circovirus, type A or B, having, after alignment and comparison with the corresponding fragments of known porcine circoviruses, at least one nucleotide or base of different nature. For example, the specific nucleotide fragments of the PWD circovirus of type A can easily be determined by referring to Figure 3 of the present invention in which the nucleotides or bases of the sequence SEQ ID No. 1 (circopordfp) are shown which are of different nature, after alignment of said sequence SEQ ID No. 1 with the other two sequences of known porcine circovirus (circopormeeh and circopormank).

Homologous nucleotide sequence in the sense of the present invention is understood as meaning a nucleotide sequence having at least a percentage identity with the bases of a nucleotide sequence according to the invention of at least 80%, preferably 90% or 95%, this percentage being purely statistical and it being possible to distribute the differences between the two nucleotide sequences at random and over the whole of their length.

Specific homologous nucleotide sequence in the sense of the present invention is understood as meaning a homologous nucleotide sequence having at least one nucleotide sequence of a specific fragment, such as defined above. Said "specific" homologous sequences can comprise, for example, the sequences corresponding to the genomic sequence or to the sequences of its fragments representative of variants of PWD circovirus of type A or B. These specific homologous sequences can thus correspond to variations linked to mutations within strains of PWD circovirus of type A and B, and especially correspond to truncations, substitutions, deletions and/or additions of at least one nucleotide. Said homologous sequences can likewise correspond to variations linked to the degeneracy of the genetic code.

The term "degree or percentage of sequence homology" refers to "degree or percentage of sequence identity between two sequences after optimal alignment" as defined in the present application.

Two amino-acids or nucleotidic sequences are said to be "identical" if the sequence of amino-acids or nucleotidic residues, in the two sequences is the same when aligned for maximum correspondence as described below. Sequence comparisons between two (or more) peptides or polynucleotides are typically performed by comparing sequences of two optimally aligned sequences over a segment or "comparison window" to identify and compare local regions of sequence similarity. Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman, *Ad. App. Math* 2: 482 (1981), by the homology alignment algorithm of Neddleman and Wunsch, J. *Mol. Biol.* 48: 443 (1970), by the search for similarity method of Pearson and Lipman, *Proc. Natl. Acad. Sci.* (U.S.A.) 85: 2444 (1988), by computerized implementation of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by visual inspection.

"Percentage of sequence identity" (or degree or identity) is determined by comparing two optimally aligned sequences over a comparison window, where the portion of the peptide or polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) **for optimal alignment** of the two sequences. The percentage is calculated by determining the number of positions at which the identical amino-acid residue or nucleic acid base occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.

The definition of sequence identity given above is the definition that would use one of skill in the art. The definition by itself does not need the help of any algorithm, said algorithms being helpful only to achieve the optimal alignments of sequences, rather than the calculation of sequence identity.

From the definition given above, it follows that there is a well defined and only one value for the sequence identity between two compared sequences which value corresponds to the value obtained for the best or optimal alignment.

In the BLAST N or BLAST P "BLAST 2 sequence", software which is available in the web site http://www.ncbi.nlm.nih.gov/gorf/bl2.html, and habitually used by the inventors and in general by the skilled man for comparing and determining the identity between two sequences, gap cost which depends on the sequence length to be compared is directly selected by the software (i.e. 11.2 for substitution matrix BLOSUM-62 for length > 85).

In the present description, PWD circovirus will be understood as designating the circoviruses associated with piglet weight loss disease (PWD) of type A (PCVA) or type B (PCVB), defined below by their genomic sequence, as well as the circoviruses whose nucleic sequences are homologous to the sequences of PWD circoviruses of type A or B, such as in particular the circoviruses corresponding to variants of the type A or of the type B.

Complementary nucleotide sequence of a sequence of the invention is understood as meaning any DNA whose nucleotides are complementary to those of the sequence of the invention, and whose orientation is reversed (antiparallel sequence).

Hybridization under conditions of stringency with a nucleotide sequence according to the invention is understood as meaning a hybridization under conditions of temperature and ionic strength chosen in such a way that they allow the maintenance of the hybridization between two fragments of complementary DNA.

By way of illustration, conditions of great stringency of the hybridization step with the aim of defining the nucleotide fragments described above are advantageously the following.

The hybridization is carried out at a preferential temperature of 65°C in the presence of SSC buffer, 1 × SSC corresponding to 0.15 M NaCl and 0.05 M Na citrate. The washing steps, for example, can be the following:

- 2 × SSC, at ambient temperature followed by two washes with 2 × SSC, 0.5% SDS at 65°C; 2 × 0.5 × SSC, 0.5% SDS; at 65°C for 10 minutes each.

The conditions of intermediate stringency, using, for example, a temperature of 42°C in the presence of a 2 × SSC buffer, or of less stringency, for example a temperature of 37°C in the presence of a 2 × SSC buffer, respectively require a globally less significant complementarity for the hybridization between the two sequences.

The stringent hybridization conditions described above for a polynucleotide with a size of approximately 350 bases will be adapted by the person skilled in the art for oligonucleotides of greater or smaller size, according to the teaching of Sambrook et al., 1989.

Among the nucleotide sequences according to the invention, those are likewise preferred which can be used as a primer or probe in methods allowing the homologous sequences according to the invention to be obtained, these methods, such as the polymerase chain reaction (PCR), nucleic acid cloning and sequencing, being well known to the person skilled in the art.

Among said nucleotide sequences according to the invention, those are again preferred which can be used as a primer or probe in methods allowing the presence of PWD circovirus or one of its variants such as defined below to be diagnosed.

The nucleotide sequences according to the invention capable of modulating, of inhibiting or of inducing the expression of PWD circovirus gene, and/or capable of modulating the replication cycle of PWD circovirus in the host cell and/or organism are likewise preferred. Replication cycle will be understood as designating the invasion and the multiplication of PWD circovirus, and its propagation from host cell to host cell in the host organism.

Among said nucleotide sequences according to the invention, those corresponding to open reading frames, called ORF sequences, and coding for polypeptides, such as, for example, the sequences SEQ ID No. ~~{3}~~ **[9]** (ORF1),

SEQ ID No. ~~{4}~~ **[11]** (ORF2) and SEQ ID No. ~~{5}~~ **[13]** (ORF3) respectively corresponding to the nucleotide sequences between the positions 47 and 985 determined with respect to the position of the nucleotides on the sequence SEQ ID No. 1, the positions 1723 and 1022 and the positions 658 and 38 with respect to the position of the nucleotides on the sequence SEQ ID No. ~~{2}~~ **[5]** (represented according to the orientation 3'→5'), the ends being included, or alternatively the sequences SEQ ID No. ~~{11}~~ **[23]** (ORF'1), SEQ ID No. ~~{12}~~ **[25]** (ORF'2) and SEQ ID No. ~~{13}~~ **[27]** (ORF'3), respectively corresponding to the sequences between the positions 51 and 995 determined with respect to the position of the nucleotides on the sequence SEQ ID No. ~~{9}~~ **[15]**, the positions 1734 and 1033 and the positions 670 and 357, the positions being determined with respect to the position of the nucleotides on the sequence SEQ ID No. ~~{10}~~ **[19]** (represented according to the orientation 3'→5'), the ends being included, are finally preferred.

The nucleotide sequence fragments according to the invention can be obtained, for example, by specific amplification, such as PCR, or after digestion with appropriate restriction enzymes of nucleotide sequences according to the invention, these methods in particular being described in the work of Sambrook et al., 1989. Said representative fragments can likewise be obtained by chemical synthesis when their size is not very large and according to methods well known to persons skilled in the art.

Modified nucleotide sequence will be understood as meaning any nucleotide sequence obtained by mutagenesis according to techniques well known to the person skilled in the art, and containing modifications with respect to the normal sequences according to the invention, for example mutations in the regulatory and/or promoter sequences of polypeptide expression, especially leading to a modification of the rate of expression of said polypeptide or to a modulation of the replicative cycle.

Modified nucleotide sequence will likewise be understood as meaning any nucleotide sequence coding for a modified polypeptide such as defined below.

The present invention relates to nucleotide sequences of PWD circovirus according to the invention, characterized in that they are selected from the sequences SEQ ID No. ~~{3, SEQ ID No. 4, SEQ ID No. 5}~~ **[9]**, SEQ ID No. 11, SEQ ID No. ~~{12}~~ **[13]**, SEQ ID No. ~~{13}~~ **[23, SEQ ID No. 25, SEQ ID No. 27]** or one of their fragments.

The invention likewise relates to nucleotide sequences characterized in that they comprise a nucleotide sequence selected from:

a) a nucleotide sequence SEQ ID No. ~~{3, SEQ ID No. 4, SEQ ID No. 5}~~ **[9]**, SEQ ID No. 11, SEQ ID No. ~~{12}~~ **[13]**, SEQ ID No. ~~{13}~~ **[23, SEQ ID No. 25, SEQ ID No. 27]** or one of their fragments;

b) a nucleotide sequence of a specific fragment of a sequence such as defined in a);

c) a homologous nucleotide sequence having at least 80% identity with a sequence such as defined in a) or b);

d) a complementary nucleotide sequence or sequence of RNA corresponding to a sequence such as defined in a), b) or c); and

e) a nucleotide sequence modified by a sequence such as defined in a), b), c) or d).

As far as homology with the nucleotide sequences SEQ ID No. ~~{3, SEQ ID No. 4, SEQ ID No. 5}~~ **[9]**, SEQ ID No. 11, SEQ ID No. ~~{12}~~ **[13]**, SEQ ID No. ~~{13}~~ **[23, SEQ ID No. 25, SEQ ID No. 27]** or one of their fragments is concerned, the homologous, especially specific, sequences having a percentage identity with one of the sequences SEQ ID No. ~~{3, SEQ ID No. 4, SEQ ID No. 5}~~ **[9]**, SEQ ID No. 11, SEQ ID No. ~~{12}~~ **[13]**, SEQ ID No. ~~{13}~~ **[23, SEQ ID No. 25, SEQ ID No. 27]** or one of their fragments of at least 80%, preferably 90% or 95%, are preferred. Said specific homologous sequences can comprise, for example, the sequences corresponding to the sequences ORF1, ORF2, ORF3, ORF'1, ORF'2 and ORF'3 of PWD circovirus variants of type A or of type B. In the same manner, these specific homologous sequences can correspond to variations linked to

mutations within strains of PWD circovirus of type A or of type B and especially correspond to truncations, substitutions, deletions and/or additions of at least one nucleotide.

Among nucleotide sequences according to the invention, the sequence SEQ ID No. ~~{11}~~ **[23]** which has a homology having more than 80% identity with the sequence SEQ ID No. ~~{3}~~ **[9]**, as well as the sequence SEQ ID No. ~~{12}~~ **[25]**, are especially preferred.

Preferably, the invention relates to the nucleotide sequences according to the invention, characterized in that they comprise a nucleotide sequence selected from the following sequences:

a) **[SEQ ID No. 33]** 170 5' TGTGGCGA 3';

b) **[SEQ ID No. 34]** 450 5' AGTTTCCT 3';

c) **[SEQ ID No. 35]** 1026 5' TCATTTAGAGGGTCTTTCAG 3';

d) **[SEQ ID No. 36]** 1074 5' GTCAACCT 3';

e) **[SEQ ID No. 37]** 1101 5' GTGGTTGC 3';

f) **[SEQ ID No. 38]** 1123 5' AGCCCAGG 3';

g) **[SEQ ID No. 39]** 1192 5' TTGGCTGG 3';

h) **[SEQ ID No. 40]** 1218 5' TCTAGCTCTGGT 3';

i) **[SEQ ID No. 41]** 1501 5' ATCTCAGCTCGT 3';

j) **[SEQ ID No. 42]** 1536 5' TGTCCTCCTCTT 3';

k) **[SEQ ID No. 43]** 1563 5' TCTCTAGA 3';

l) **[SEQ ID No. 44]** 1623 5' TGTACCAA 3';

m) **[SEQ ID No. 45]** 1686 5' TCCGTCTT 3';

and their complementary sequences.

In the list of nucleotide sequences a)-m) above, the underlined nucleotides are mutated with respect to the two known sequences of circovirus which are nonpathogenic to pigs. The number preceding the nucleotide sequence represents the position of the first nucleotide of said sequence in the sequence SEQ ID No. 1.

The invention comprises the polypeptides encoded by a nucleotide sequence according to the invention, preferably a polypeptide whose sequence is represented by a fragment, especially a specific fragment, of one of the six sequences of amino acids represented in Figure 2, these six amino acid sequences corresponding to the polypeptides which can be encoded according to one of the three possible reading frames of the sequence SEQ ID No. 1 or of the sequence SEQ ID No. ~~{2}~~ **[5]**, or a polypeptide whose sequence is represented by a fragment, especially a specific fragment, of one of the six sequences of amino acids shown in Figure 8, these six sequences of amino acids corresponding to the polypeptides which can be encoded according to one of the three possible reading frames of the sequence SEQ ID No. ~~{9}~~ **[15]** or of the sequence SEQ ID No. ~~{10}~~ **[19]**.

The invention likewise relates to the polypeptides, characterized in that they comprise a polypeptide selected from the amino acid sequences SEQ ID No. ~~{6}~~ **[10]**, SEQ ID No. ~~{7, SEQ ID No. 8}~~ **[12]**, SEQ ID No. 14, SEQ ID No. ~~{15}~~ **[24]**, SEQ ID No. ~~{16}~~ **[26, SEQ ID No. 28]** or one of their fragments.

Among the polypeptides according to the invention, the polypeptide of amino acid sequence SEQ ID No. ~~{14}~~ **[24]** which has a homology having more than 80% identity with the sequence SEQ ID No. ~~{6}~~ **[10]**, as well as the polypeptide of sequence SEQ ID No. ~~{15}~~ **[26]**, are especially preferred.

The invention also relates to the polypeptides, characterized in that they comprise a polypeptide selected from:

a) a specific fragment of at least 5 amino acids of a polypeptide of an amino acid sequence according to the invention;

b) a polypeptide homologous to a polypeptide such as defined in a);

c) a specific biologically active fragment of a polypeptide such as defined in a) or b); and

d) a polypeptide modified by a polypeptide such as defined in a), b) or c).

Among the polypeptides according to the invention, the polypeptides of amino acid sequences SEQ ID No. ~~{17}~~ **[29]**, SEQ ID No. ~~{18}~~ **[30]**, SEQ ID No. ~~{19}~~ **[31]** and SEQ ID No. ~~{20}~~ **[32]** are also preferred, these polypeptides being especially capable of specifically recognizing the antibodies produced during infection by the PWD circovirus of type B. These polypeptides thus have epitopes specific for the PWD circovirus of type B and can thus be used in particular in the diagnostic field or as immunogenic agent to confer protection in pigs against infection by PWD circovirus, especially of type B.

In the present description, the terms polypeptide, peptide and protein are interchangeable.

It must be understood that the invention does not relate to the polypeptides in natural form, that is to say that they are not taken in their natural environment but that they can be isolated or obtained by purification from natural sources, or else obtained by genetic recombination, or alternatively by chemical synthesis and that they can thus contain unnatural amino acids, as will be described below.

Polypeptide fragment according to the invention is understood as designating a polypeptide containing at least 5 consecutive amino acids, preferably 10 consecutive amino acids or 15 consecutive amino acids.

In the present invention, specific polypeptide fragment is understood as designating the consecutive polypeptide fragment encoded by a specific fragment nucleotide sequence according to the invention.

Homologous polypeptide will be understood as designating the polypeptides having, with respect to the natural polypeptide, certain modifications such as, in particular, a deletion, addition or substitution of at least one amino acid, a truncation, a prolongation, a chimeric fusion, and/or a mutation. Among the homologous polypeptides, those are preferred whose amino acid sequence has at least 80%, preferably 90%, homology with the sequences of amino acids of polypeptides according to the invention.

Specific homologous polypeptide will be understood as designating the homologous polypeptides such as defined above and having a specific fragment of polypeptide according to the invention.

In the case of a substitution, one or more consecutive or nonconsecutive amino acids are replaced by "equivalent" amino acids. The expression "equivalent" amino acid is directed here at designating any amino acid capable of being substituted by one of the amino acids of the base structure without, however, essentially modifying the biological activities of the corresponding peptides and such that they will be defined by the following.

These equivalent amino acids can be determined either by depending on their structural homology with the amino acids which they substitute, or on results of comparative tests of biological activity between the different polypeptides, which are capable of being carried out.

By way of example, the possibilities of substitutions capable of being carried out without resulting in an extensive modification of the biological activity of the corresponding modified polypeptides will be mentioned, the replacement, for example, of leucine by valine or isoleucine, of aspartic acid by glutamic acid, of glutamine by asparagine, of arginine by lysine etc., the reverse substitutions naturally being envisageable under the same conditions.

The specific homologous polypeptides likewise correspond to polypeptides encoded by the specific homologous nucleotide sequences such as defined above and thus comprise in the present definition the polypeptides which are mutated or correspond to variants which can exist in PWD circovirus, and which especially correspond to truncations, substitutions, deletions and/or additions of at least one amino acid residue.

Specific biologically active fragment of a polypeptide according to the invention will be understood in particular as designating a specific polypeptide fragment, such as defined above, having at least one of the characteristics of polypeptides according to the invention, especially in that it is: